

***Picea pungens* (Pinaceae) branch and trunk wood essential oil profile comparison from cultivated trees in northern Idaho (USA)**

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ABSTRACT

Picea pungens is an essential oil-bearing plant in the Pinaceae family. The essential oil of *P. pungens* was produced through steam distillation and examined to identify and compare the volatile profiles of both the branch and trunk wood. The resulting samples ($n = 12$) were analyzed by GC/MS and GC/FID. Prominent (defined as avg. $\geq 2\%$) volatile compounds from *P. pungens* branch include (averages) α -pinene (29.0%), camphene (12.3%), β -pinene (6.6%), myrcene (5.5%), δ -3-carene (7.9%), limonene (20.2%), camphor (4.2%), and bornyl acetate (2.3%). Prominent (defined as avg. $\geq 2\%$) volatile compounds from trunk wood include (averages) α -pinene (60.3%), camphene (2.0%), β -pinene (16.2%), myrcene (3.0), δ -3-carene (3.1%), and limonene (8.3%). Comparing the branch and trunk wood essential oil profiles, they are similar, with six prominent volatile compounds in common. However, the different parts of the tree have specific compounds unique to that portion of the tree and could be used for determining quality of commercial essential oil and for chemotaxonomic identification. Published online www.phytologia.org *Phytologia* 105(1): 18-24 (March 21, 2023). ISSN 030319430.

KEY WORDS: aromatic profile, branch, chemotaxonomy, essential oil, *Picea pungens*, trunk

Picea pungens Engelm. (blue spruce) is a conifer in the Pinaceae family with a native range primarily in the western United States and southern Rocky Mountains, with populations growing between 1800 m to 3000 m elevation (Flora of North America 1993; World Flora Online 2023). Trees in their native habitat typically grow to a height of 50 m, with trunk diameters of 1.5 m, prickly and stiff and bluish green needles that spread radially (Fig. 1) (Auders & Spicer 1990; Bailey 1951; Flora of North America 1993). *Picea pungens* is commonly cultivated and distributed under various names of cultivars. They are primarily valued for ornamental purposes due to their pyramidal, symmetrical shape, unique color, and are not desirable as timber due to wood knots. (Auders & Spicer 1990; Welch 1979).



Figure 1. Botanical illustration showing portion of *Picea pungens* limb with mature seed cone. Illustrated by Zach Nielsen, Utah Valley University (Orem, UT, USA).

Previous research on *P. pungens* has shown the stem portion of the tree to be primarily composed of the following compounds and relative abundances: α -pinene (41.0%), camphene (2.4%), β -pinene (22.7%), sabinene (2.5%), δ -3-carene (24.7%), limonene (4.7%) (Lewinsohn et al. 1991). Other research that differentiated foliage and xylem/bark chemical profiles showed that camphene (12.6%), myrcene (9.8%), limonene (30.9%), camphor (11.2%) and bornyl acetate (14.0%) all had higher concentrations in the foliage than in the xylem/bark. In contrast, the same study showed that α -pinene (71.4%, xylem), β -pinene (20.0%, xylem), sabinene (2.4%, xylem), δ -3-carene (22.2%, bark) were more abundant in the xylem/bark (Hanover 1992). Other researchers confirmed that primary constituents of *P. pungens* included α -pinene, camphene, sabinene, β -pinene, myrcene, δ -3-carene, and limonene throughout the tree (Ott et al. 2021; Raber et al. 2021).

Previous research on evergreen species in the Pinaceae family has shown that trunk wood essential oil often contains unique compounds that can be used for chemotaxonomic investigations (Poulson et al. 2020; Ruggles et al. 2022). The current study establishes essential oil profiles for samples extracted from the branch and trunk wood of *P. pungens* and provides integrative tools for determining quality of commercial essential oil and for chemotaxonomic investigations.

MATERIALS AND METHODS

Picea pungens branch and trunk plant material were collected from privately owned cultivated tree farmland in Bonner County, Idaho, USA (48°34'41.3" N 116°26'16.1" W; 696 m elevation). The plant material was collected on the following dates with the corresponding average daily temperatures (for simplicity and consistency, essential oil samples are referred to by a letter, A-L) (historical weather data from ncei.noaa.gov): (A/G: March 24, 2021, 5.3°C), (B/H: April 19, 2021, 11.7°C), (C/I: July 8, 2021, 23.9°C), (D/J: September 20, 2021, 10.8°C), (E/K: November 8, 2021, 3.9°C), (F/L: December 7, 2021, -6.1°C). A total of eighteen trees (avg. age of eight years – determined by dendrochronology) were cut approximately halfway up the trunk utilizing the stump culture technique (Wunderlich 2020). Both the branches and the trunk material were used for this research (Fig. 2). The branches include limb material and needles. The trunk material includes the inner and outer bark, cambium, sapwood, and heartwood sections. Representative voucher samples used for identification are held in the University of Idaho Stillinger Herbarium in Moscow, ID, USA, and the Consortium of Pacific Northwest Herbaria in Seattle, WA, USA.



Figure 2. Botanical illustration showing (from left to right) *Picea pungens* tree (approximate age 8 years – young tree without seed cones), chipped branch plant material, and chipped trunk plant material. Illustrated by Zach Nielsen, Utah Valley University (Orem, UT, USA).

The plant material was prepared for distillation as follows (Figure 1). The limbs were removed flush against the trunks and separated. The branch material included all limb material including needles. The trunk material was the main tree trunk with no needles or limb material. The different plant materials were chipped with a woodchipper, blended, and stored in an airtight container at -20 ± 2 °C until steam distilled. Six separate steam distillations were performed on the prepared chips for each portion, resulting in a total of twelve distillations for this study. The distillations were conducted in a 12-L food grade stainless steel distillation chamber (Albrigi Luigi S.R.L., Italy) with approximately 2.5 liters of water added to the chamber. Steam was passed through suspended chips for two hours after pass-over and the essential oil was separated from hydrosol using a cooling condenser and collected in an analytical graduated cylinder. The essential oil was stored in a sealed amber glass bottle until analysis.

Essential oil samples were analyzed, and volatile compounds identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 0.25 mm \times 60 m, 0.25 μ m film thickness, fused silica capillary column. Operating conditions: 0.1 μ L of sample (20% soln. for essential oils in ethanol), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min., oven ramp rate of 4.5 °C per min. to 310 °C with a hold time of 5 min. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per sec., source temp. 230 °C, and quadrupole temp. 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams 2007) using Chemstation library search in conjunction with retention indices. Note that limonene/ β -phellandrene elutes as single peaks. Their amounts were determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β -phellandrene). Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B GC and Agilent J&W DB-5, 0.25 mm \times 60 m, 0.25 μ m film thickness, fused silica capillary column. Operating conditions: 0.1 μ L of sample (20% soln. for essential oils in ethanol, 1% for reference compounds in ethanol, 0.1% soln. for C7–C30 alkanes in hexane), 25:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 2 min., oven ramp rate of 3.0 °C per min. to 250 °C with a hold time of 3 min. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were identified using retention indices coupled with retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

RESULTS AND DISCUSSION

The essential oil profile of both branch and trunk plant material of *Picea pungens* were determined by GC/MS and GC/FID (Table 1). Prominent (defined as avg. $\geq 2\%$) volatile compounds from *P. pungens* branch include (averages) α -pinene (29.0%), camphene (12.3%), β -pinene (6.6%), myrcene (5.5%), δ -3-carene (7.9%), limonene (20.2%), camphor (4.2%), and bornyl acetate (2.3%). Prominent (defined as avg. $\geq 2\%$) volatile compounds from trunk wood include (averages) α -pinene (60.3%), camphene (2.0%), β -pinene (16.2%), myrcene (3.0%), δ -3-carene (3.1%), and limonene (8.3%). These types of similarities seen in essential oil profiles of different plant parts of the same species may be a defining quality of plants of the Pinaceae family (Poulson et al. 2020; Ruggles et al. 2022).

In comparison, the essential oil profile of both the branches and trunk wood of *P. pungens* are similar, sharing six prominent (defined as avg. $\geq 2\%$) volatile compounds in common, including α -pinene, camphene, β -pinene, myrcene, δ -3-carene, and limonene. However, the values (averages) differ greatly with relative percent differences (Table 2) ranging from 58.8% (myrcene) to 144.1% (camphene). The relative abundance of camphene, myrcene, δ -3-carene, limonene, and bornyl acetate were greater in the branch material, whereas α -pinene, sabinene, and β -pinene were greater in the trunk. The following compounds (averages) were found in both plant parts but detected in higher relative abundance in the branches, compared to the trunk: 1,8-cineole (1.6%, 0.1%), camphor (4.2%, 0.4%), camphene hydrate (1.3%, 0.1%), and borneol (1.0%, 0.1%), respectively. Several minor compounds differentiate the branch and trunk essential oil profiles from each other. Hexanal, (3Z)-hexenol, n-hexanol, ethyl hexanoate, 3-

methyl-3-butenyl-3-methyl-butanoate, isoborneol, ethyl octanoate, carvone, piperitone, and α -muurolene were all found in the branches, but not in the trunk. Whereas α -pinene oxide, trans-pinocamphone, cryptone, β -elemene, and germacrene D were all detected in the trunk, but not in the branches. Characteristic compounds such as those have been used to identify and differentiate plant species on trees burnt in wildfires when traditional taxonomic methods could not be used (Wilson et al. 2021). Additionally, both the presence and abundance of unique compounds can be used for determination of the quality of a commercial essential oil product (i.e., provide insight into the harvest details and distillation method of an aromatic plant) (Wilson et al. 2021).

Table 1. Aromatic profile of *Picea pungens* essential oil from both branch and trunk material. Compounds not detected in a sample are denoted as not detected (ND) and those with values less than 0.1% are denoted as traces (t). Compounds less than 1.0% that were unidentified are not included. KI is the Kovat's Index using a linear calculation on the DB-5 column (Adams 2007). Relative area percent is determined by GC-FID. All essential oil samples were analyzed in triplicate to ensure repeatability (SD <1).

Compound	KI	Branch						Trunk					
		A	B	C	D	E	F	G	H	I	J	K	L
hexanal	801	nd	t	t	t	t	nd	nd	nd	nd	nd	nd	nd
(3Z)-hexenol	850	0.1	t	t	0.2	0.1	nd	nd	nd	nd	nd	nd	nd
n-hexanol	863	t	nd	nd	t	t	nd	nd	nd	nd	nd	nd	nd
santene	884	0.6	0.2	0.3	0.2	0.3	0.3	0.2	0.1	0.1	0.1	0.3	0.1
tricyclene	921	1.6	1.1	1.7	1.7	1.4	1.8	0.6	0.6	0.6	0.7	0.6	0.5
α -thujene	924	0.2	0.2	0.1	0.2	0.1	0.3	0.3	0.2	0.2	0.3	0.2	0.3
α -pinene	932	24.2	43.6	22.1	32.3	24.1	27.5	50.3	71.5	51.0	65.6	63.5	59.9
camphene	946	12.5	8.0	14.4	13.3	11.5	14.4	2.6	2.3	1.8	2.0	2.0	1.5
thuja-2,4(10)-diene	953	t	t	t	t	t	t	t	0.1	t	t	0.1	t
sabinene	969	1.2	1.1	0.9	0.8	0.5	0.8	2.3	1.2	1.2	1.0	0.9	1.3
β -pinene	974	8.5	4.4	8.3	5.7	5.3	7.6	19.0	6.4	25.6	13.2	14.9	18.1
myrcene	988	7.0	4.7	6.8	6.2	5.3	3.0	3.7	2.1	2.6	2.7	3.8	2.9
ethyl hexanoate	997	t	0.1	t	nd	0.1	t	nd	nd	nd	nd	nd	nd
α -phellandrene	1002	t	t	t	t	t	t	t	t	t	t	t	t
δ -3-carene	1008	9.3	10.4	8.0	8.4	3.8	7.7	5.2	2.8	2.5	3.5	3.1	1.3
α -terpinene	1014	t	t	t	t	t	t	t	t	t	t	t	nd
p-cymene	1020	t	t	t	t	0.1	t	t	t	t	t	t	t
o-cymene	1022	0.5	0.5	0.5	0.6	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.4
limonene	1024	20.7	17.1	24.8	16.2	20.9	21.3	10.3	7.5	8.8	7.0	7.2	8.7
β -phellandrene	1025	0.4	0.2	0.3	0.4	0.1	0.6	0.8	0.3	0.4	0.4	0.4	0.2
1,8-cineole	1026	1.8	1.0	2.4	1.3	1.1	1.7	0.3	0.2	0.1	0.1	t	0.1
(Z)- β -ocimene	1032	t	0.1	t	t	t	t	0.1	0.1	t	0.1	0.1	0.1
(E)- β -ocimene	1044	0.1	0.1	t	t	t	0.1	t	t	t	t	t	nd
γ -terpinene	1054	t	t	t	t	t	t	t	0.1	t	t	t	t
p-mentha-2,4(8)-diene	1085	t	t	t	t	t	t	t	t	t	t	t	t
terpinolene	1086	0.4	0.4	0.4	0.2	0.3	0.5	0.3	0.4	0.1	0.2	0.2	0.1
linalool	1095	t	t	t	t	0.1	t	t	0.1	t	t	t	t
α -pinene oxide	1099	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	t	t
3-methyl-3-butenyl-3-methyl-butanoate	1112	nd	t	nd	nd	0.1	t	nd	nd	nd	nd	nd	nd
endo-fenchol	1114	t	t	t	t	0.1	t	t	t	t	t	t	t
α -campholenal	1122	0.1	t	0.1	0.1	0.1	t	t	t	0.1	t	t	0.1
trans-pinocarveol	1135	t	t	t	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2
camphor	1141	3.1	2.5	2.3	4.2	8.1	4.8	0.7	0.6	0.3	0.2	0.2	0.3
camphene hydrate	1145	1.1	0.7	0.8	1.4	2.2	1.7	0.2	0.2	0.1	0.1	t	0.1
isoborneol	1155	t	t	0.1	0.1	0.1	t	nd	nd	nd	nd	nd	nd
trans-pinocamphone	1158	nd	nd	nd	nd	nd	nd	0.1	t	0.1	t	t	0.1
borneol	1165	0.9	0.4	0.7	1.0	1.9	1.0	0.2	0.2	0.2	0.1	0.1	0.1
cis-pinocamphone	1172	t	nd	t	nd	nd	nd	t	t	t	t	t	t

Compound	KI	Branch						Trunk					
		A	B	C	D	E	F	G	H	I	J	K	L
terpinen-4-ol	1174	0.2	0.2	0.2	0.3	0.5	0.2	0.1	0.2	0.2	0.1	0.1	0.2
p-cymen-8-ol	1179	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2
cryptone	1183	nd	nd	nd	nd	nd	nd	nd	nd	0.1	t	nd	0.1
α -terpineol	1186	0.3	0.2	0.3	0.3	0.6	0.3	0.1	0.1	0.1	0.1	0.1	0.1
methyl chavicol	1195	0.1	t	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2
ethyl octanoate	1196	t	t	t	t	t	nd	nd	nd	nd	nd	nd	nd
verbenone	1204	t	t	nd	t	0.1	t	t	t	0.1	t	t	0.1
trans-carveol	1215	t	t	t	t	0.2	t	t	t	t	t	t	0.1
citronellol	1223	0.1	0.1	0.1	0.1	0.5	0.1	t	t	0.1	t	t	0.1
thymol methyl ether	1232	t	t	t	t	t	t	t	t	t	t	t	t
carvone	1239	nd	t	t	0.1	0.2	t	nd	nd	nd	nd	nd	nd
piperitone	1249	t	nd	0.1	t	t	t	nd	nd	nd	nd	nd	nd
bornyl acetate	1284	2.6	0.9	2.8	1.9	3.4	2.3	0.7	0.9	1.4	0.8	0.4	1.0
citronellyl acetate	1350	t	nd	nd	nd	nd	nd	nd	nd	nd	t	t	t
β -elemene	1389	nd	nd	nd	nd	nd	nd	t	t	0.1	t	t	t
(E)-caryophyllene	1417	t	t	t	t	t	t	t	t	t	t	t	t
germacrene D	1480	nd	nd	nd	nd	nd	nd	nd	t	nd	t	t	nd
α -muurolene	1500	nd	nd	t	t	t	t	nd	nd	nd	nd	nd	nd
cubebol	1514	nd	nd	0.1	0.1	t	0.1	t	t	t	t	t	t
δ -cadinene	1522	nd	t	t	0.1	t	t	t	t	t	t	0.1	t
cembrene	1937	nd	t	t	t	t	t	t	t	t	t	t	t

Table 2. The relative area % of prominent (defined as avg. $\geq 2\%$) compounds in *Picea pungens* essential oil, averaged across all samples. The relative percent difference (RPD) is provided.

Shared Prominent Compounds	Branches (avg.)	Trunk (avg.)	RPD
α -pinene	29.0	60.3	70.1
camphene	12.3	2.0	144.1
β -pinene	6.6	16.2	84.2
myrcene	5.5	3.0	58.8
δ -3-carene	7.9	3.1	87.3
limonene	20.2	8.3	83.5

The plant material was harvested throughout the year (dates ranging from March to December) in temperatures varying from -6.1°C to 23.9°C . While relative abundance of volatile compounds vary from sample to sample, this does not appear to be greatly influenced by the season or temperature (Fig. 3 & 4). This could allow for commercial production of *P. pungens* essential oil throughout growing and dormant seasons, while maintaining a relatively consistent product.

CONCLUSIONS

The methods used in this study have previously been used by these and other researchers. While the approach is not novel, the findings pertaining to this species are new to this field of science.

Both the compounds in common and the compounds unique to each plant part observed in this study provide a foundation for future investigations in both quality assurance of commercial production of blue spruce essential oil and for chemotaxonomic investigations. Future research performed on larger populations of blue spruce and including both cultivated and wildcrafted samples from various regions will provide more insight on these topics.

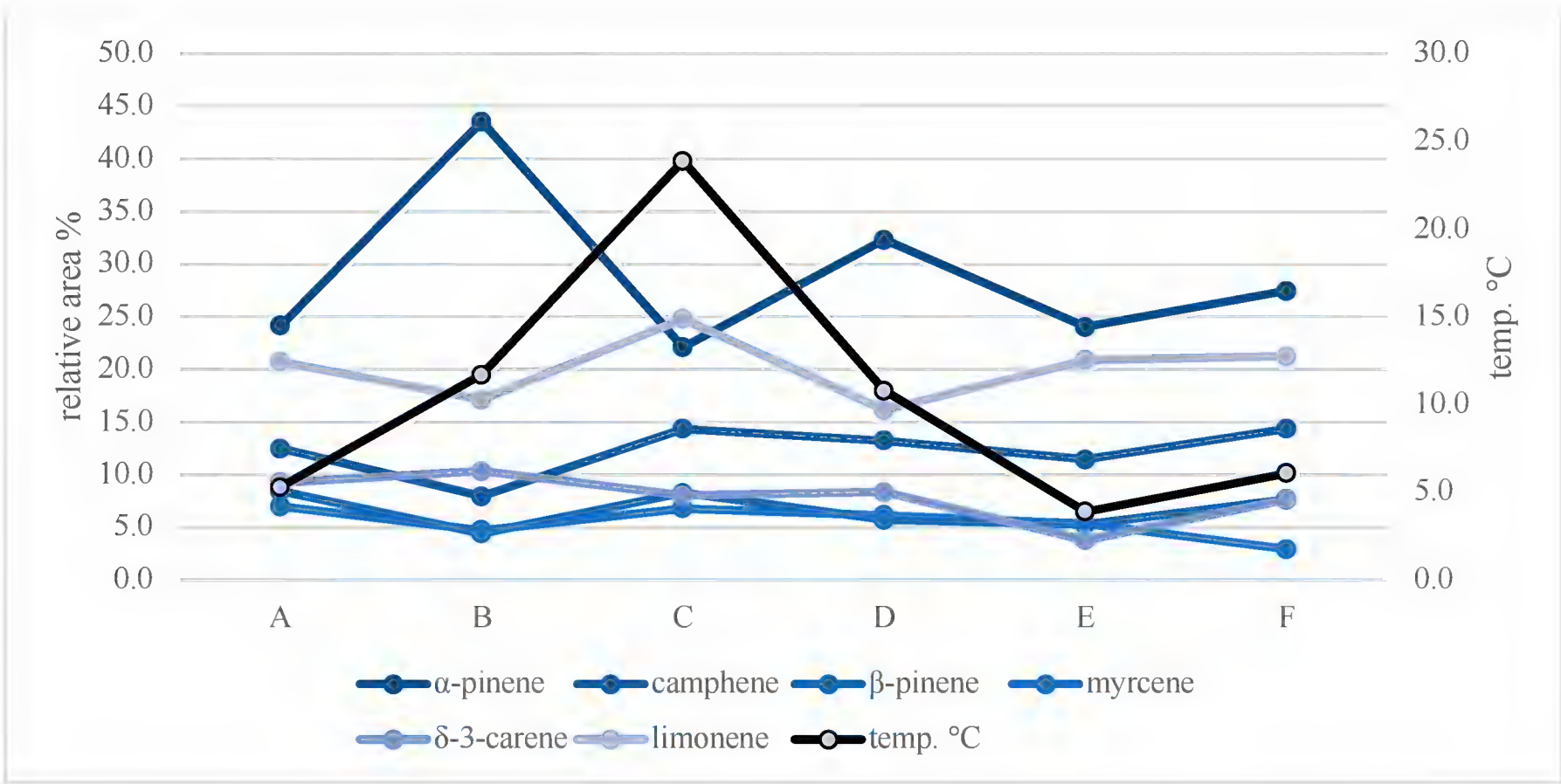


Figure 3. Chart showing relationship between relative area % (Y-axis) of volatile compounds, prominent (defined as $\geq 2\%$) compound names (X-axis), and temperature °C (Z-axis) in branch essential oil samples (A-F).

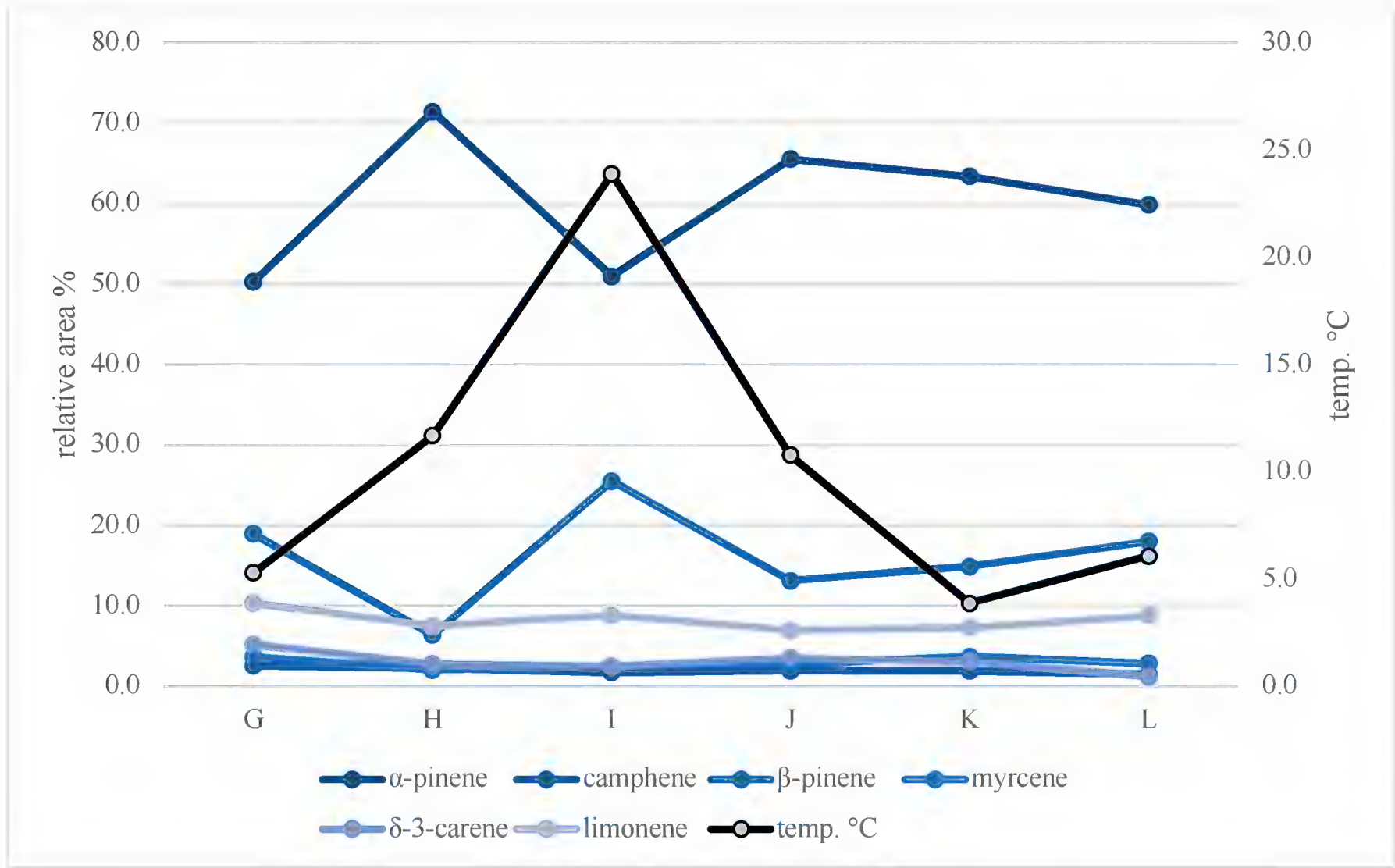


Figure 4. Chart showing relationship between relative area % (Y-axis) of volatile compounds, prominent (defined as $\geq 2\%$) compound names (X-axis), and temperature °C (Z-axis) in trunk essential oil samples (G-L).

ACKNOWLEDGEMENTS

The authors would like to thank the following individuals and organizations for their assistance with the study: Michael Carter from the Young Living Highland Flats Tree Farm and Distillery for assisting with sample collection, Zach Nielsen from Utah Valley University for the botanical illustration, and the D. Gary Young Research Institute for providing support for this project.

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